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Small Animals in the Study of Pathological Effects of of Asbestos

by Paul F. Holt*

The main pathological effects attributed to asbestos are carcinogenesis and fibrogenesis. Statistical studies have shown that asbestos workers may expect a higher morbidity not only from cancer of the lung and mesothelioma but also from cancer at other sites. Carcinomas have been reported in animals following the injection of asbestos, but the production of carcinomas by inhaled asbestos is less easy to demonstrate; most examples of experimental carcinogenesis with asbestos have been produced in rats. Rats and man react differently to asbestos in that rats do not produce asbestos bodies.

The fibrosis that follows inhalation of asbestos has been frequently described, but studies with specific pathogen free animals have shown that, like the fibrosis that may follow the inhalation of silica dust, gross fibrosis involving the production of abnormal amount of collagen probably requires the intervention of infection as well as asbestos.

Because of the difficulties encountered in the direct investigation of carcinogenesis and fibrogenesis resulting from the inhalation of asbestos, attention has been directed to the mechanisms by which the lung is able to protect itself against these fibrous dusts. While nonfibrous dusts and short fibers can be ingested by macrophages and removed via the bronchus, the long fibers that may also reach the alveolar regions may not be removed by this mechanism. The probability that a fiber may reach the alveoli depends largely on the fiber diameter and only to a small extent on the fiber length, so that, for example, fibers 100 μm long may reach the alveoli of a guinea pig. These long fibers may become coated with a ferroprotein derived from hemoglobin to form an asbestos body and, after morphological changes, the asbestos body may be broken up, the fragments ingested by macrophages and dissolved. The lung is thus cleared of asbestos. In the guinea pig lung, consolidated areas from which the asbestos has disappeared shows signs of return to normal.

This clearance mechanism is inhibited by other factors: quartz dust may almost completely inhibit asbestos body formation; tobacco smoke has a considerable effect, and even very heavy loads of carbon may act similarly.

The normal lung appears able to efficiently eliminate small loads of both nonfibrous and fibrous dust, including the carcinogenic asbestos fibers. The capacity is not unlimited, however, and when the load is heavy there is a much greater probability that fibers will not be detoxicated. In addition, other factors such as silica dust and tobacco smoke may remove the protective mechanism in the lungs.

Inhalation of asbestos necessarily implies ingestion because much of the dust load of the lung eventually reaches the gastrointestinal tract. The transfer from the lungs has been studied in animals by using radioactive asbestos dusts and following their excretion in the feces, for example *(1)*. There appear to be no animal experiments that demonstrate the penetration of the intestinal wall by these ingested particles.

Animal experiments have shown that inhaled asbestos fibers may move from the lung or trachea to other tissues. From the bronchiole, they may move into the muscular coat. Fibers have been found in the lymph nodes of guinea

*University of Reading, Reading, England.

pigs that inhaled crocidolite (Fig. 1); presumably they had passed along the lymphatics. Asbestos bodies appeared in the thyroid of a guinea pig that inhaled anthophyllite asbestos.

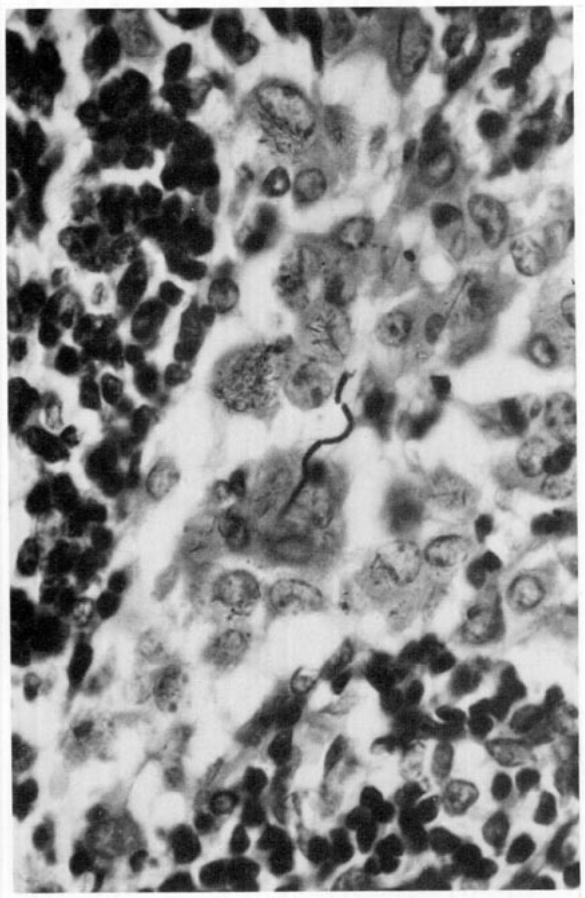

FIGURE 1. Asbestos fibers and an asbestos body in a lymph node of a guinea pig that inhaled crocidolite. Hematoxylin–eosin. 800× .

There is evidence of the movement of asbestos in the tissues in man; human beings with mesotheliomas were found to have asbestos in the lung, abdominal nodes, and peritoneum and asbestos bodies in the spleen and small bowel *(2)*.

After asbestos has been injected into animals it may be found some distance from the site of injection. Chrystotile injected into the peritoneum migrated to the diaphragm and dome of the liver *(3)*; asbestos injected into the flanks of mice was found in the spleen, liver, kidneys, brain, and lymph nodes *(4)*; chrysotile injected into the stomach of rats was found in every tissue examined *(5)*. Relatively large amounts of the asbestos dust are administered in injection experiments, and this fact and the increased pressure in the tissue may be partly responsible for the results obtained.

It is established, then, that asbestos dust that has entered the tissues by inhalation or ingestion may be found at the site of entry or at other sites. It is important to know whether these particles can be pathogenic. The main pathological effects attributed to asbestos are carcinogenesis and fibrogenesis. Experimentally there are difficulties in studying both these conditions.

Statistical studies have shown that asbestos workers may expect a higher morbidity not only from cancer of the lung and mesothelioma but also from cancer at other sites. Kogan et al. *(6)* for example, found more carcinomas of the stomach, intestine, and uterus in asbestos workers than in the general population. Deaths from all forms of cancer in asbestos workers aged 20–50 years were about one and a half times, in males over 50 years five times, and in females over 50 years 25 times those of the general population.

Carcinomas have been reported in animals following the injection of asbestos. Amosite, chrysotile, and crocidolite all produced mesotheliomas when administered by intrapleural injection *(7)*, but the production of carcinomas in animals by inhaled asbestos is less easy to demonstrate. Vorwald and his collaborators *(8)* found two squamous carcinomas of the lung in guinea pigs that had inhaled crocidolite for 1588 hr. In our experience, chrysotile *(9)* and crocidolite *(10)* inhaled by guinea pigs for shorter periods produced a marked proliferation of the bronchiolar epithelium, a condition that may precede carcinogenesis. Dutra and Carney *(11)*, for example, stated that in man a squamous metaplasia of the bronchiolar wall that occurs in asbestosis is comparable with the precancerous metaplasia of the bronchiolar columnar epithelial cells in cigarette smokers, A similar condition has not been induced in animals by ingested crocidolite. In man, carcinogenesis by asbestos usually takes some 20 years, and it is possible that the life span of small animals is too short for true

carcinogenesis by inhaled asbestos to be demonstrated.†

The fibrosis that follows the inhalation of asbestos by animals has been frequently described. Specific pathogen-free (SPF) rats and guinea pigs have been used in our later experiments, and we have not found nodular fibrosis to result from the inhalation of any type of asbestos. In earlier experiments, fibrosis was induced by inhaled asbestos in animals that had lung infections *(9, 12)*. It seems probable that gross fibrosis follows the inhalation of asbestos into an infected lung both in animals and man.

Fibrosis of the lung has been induced in SPF animals by asbestos administered by intratracheal injection, but this is probably an artifact. Gross, Harley, and De Treville *(13)* showed that even aluminum powder would cause fibrosis when administered to rats by intratracheal injection. Fibrous foci were formed in which the alveolar structure was entirely obliterated and there were coarse randomly arranged collagen bundles; The same powders did not produce fibrosis when administered by inhalation, however, although the concentration was high.

In this context the effect of silica on the lungs of animals is relevant. More than two decades ago it was established that a gross nodular fibrosis of the lung could be produced in rats and guinea pigs by silica introduced by injection or inhalation. Heppleston, Wright, and Stewart *(14)* failed to produce gross fibrosis when SPF rats inhaled silica but found a severe alveolar lipoproteinosis. Our own experiments confirm this. Dale *(15)* showed that the percentage of collagen in the lungs of quartz-injected rabbits was no greater than in controls when the lung showed no infection. Apparently gross fibrosis requires a combination of silica and infection. King, Sivalingam, and Trevella *(16)* had shown that an added infection increased the degree of fibrosis caused by silica in non-SPF rats and hematite dust, which caused little fibrosis when injected alone into the lungs of guinea pigs, produced extensive fibrosis and fatalities when injected together with tubercle bacilli *(17)*. In man, the hematological changes accompanying silicosis (elevated serum mucopolysaccharide, histamine, and hydroxypyroline values) are much greater if tuberculosis is present *(18)*, and in man no correlation between the incidence of silicosis and the duration of exposure to silica dust could be found because of the intervention of infection *(19)*.

It seems that massive fibrosis following asbestos inhalation, like the massive fibrosis following silica inhalation, requires a combination of dust and infection, and that meaningful animal experiments require SPF animals, otherwise a variable and uncontrollable factor may invalidate results. The addition of a monitored infection would allow experiments on fibrogenesis that parallel human experience. It is equally important that dusts should be administered by inhalation or ingestion to obviate the artifacts introduced by the technique of injection. If fibrogenesis cannot be regarded as the effect of asbestos alone, neither can a lipoproteinosis since quartz and even aluminum produce this effect.

These considerations indicate the difficulties that are encountered when an attempt is made to follow the pathological effects of asbestos in animals. It is because of these difficulties that we have pursued an additional line of research, studying the protective action of the tissues against asbestos and the factors that might enhance or reduce its effectiveness. In the lung alveoli and bronchioles the macrophages phagocytize dust particles. Spherical particles and very short fibers are phagocytized rapidly. The process may take many hours or may never be completed when a fiber is ingested. Macrophages containing only small particles in the cytoplasm are released into the lumen of the bronchiole and move up into the bronchus. If a macrophage contains a long fiber it may not be disposed of in this way.

During phagocytosis, the macrophage releases a subtance that induces the diapedesis of red cells from a neighboring capillary *(20)*. The red cells are ingested by the macrophage and small granules, probably ferroproteins derived from the hemoglobin are formed in the cytoplasm. They appear first to adhere to a long asbestos fiber, then to form a smooth coating on the fiber that stains blue with Perl's reagent.

†An important paper *(22)* has just appeared that describes carcinogenesis following the inhalation by 700 rats of four types of asbestos for periods up to 24 months.

Morphological changes follow *(21)*, with the production of the beaded asbestos body that eventually breaks up into fragments (Figs. 2 and 3). The fragments are taken up by other macrophages and dissolved, the released iron in the cytoplasm being demonstrable by a Prussian Blue reaction (Fig. 4). In this way long asbestos fibers are removed from the lung. If an animal inhales a small amount of asbestos, short fibers are ingested by macrophages which are removed via the bronchus; all the long fibers—or at least all the fibers visible by phase contrast microscopy—are coated within 18 months, and most of them fragment and disappear within that time. With heavier doses of asbestos, some fibers remain uncoated for long periods and presumably remain pathogenic.

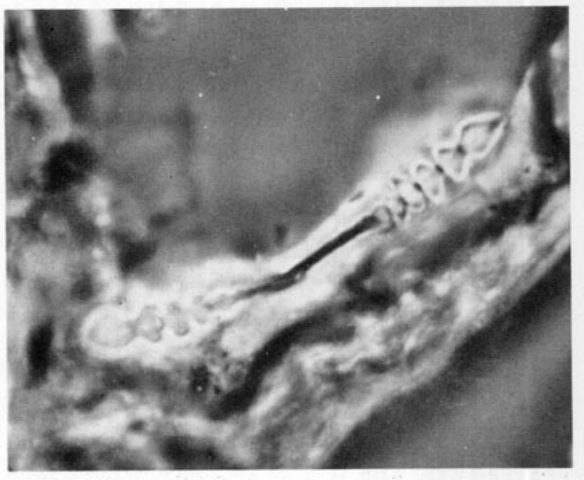

FIGURE 2. Beaded asbestos body. Guinea pig inhaled anthophyllite. Unstained; phase contrast; oil immersion. 1400×.

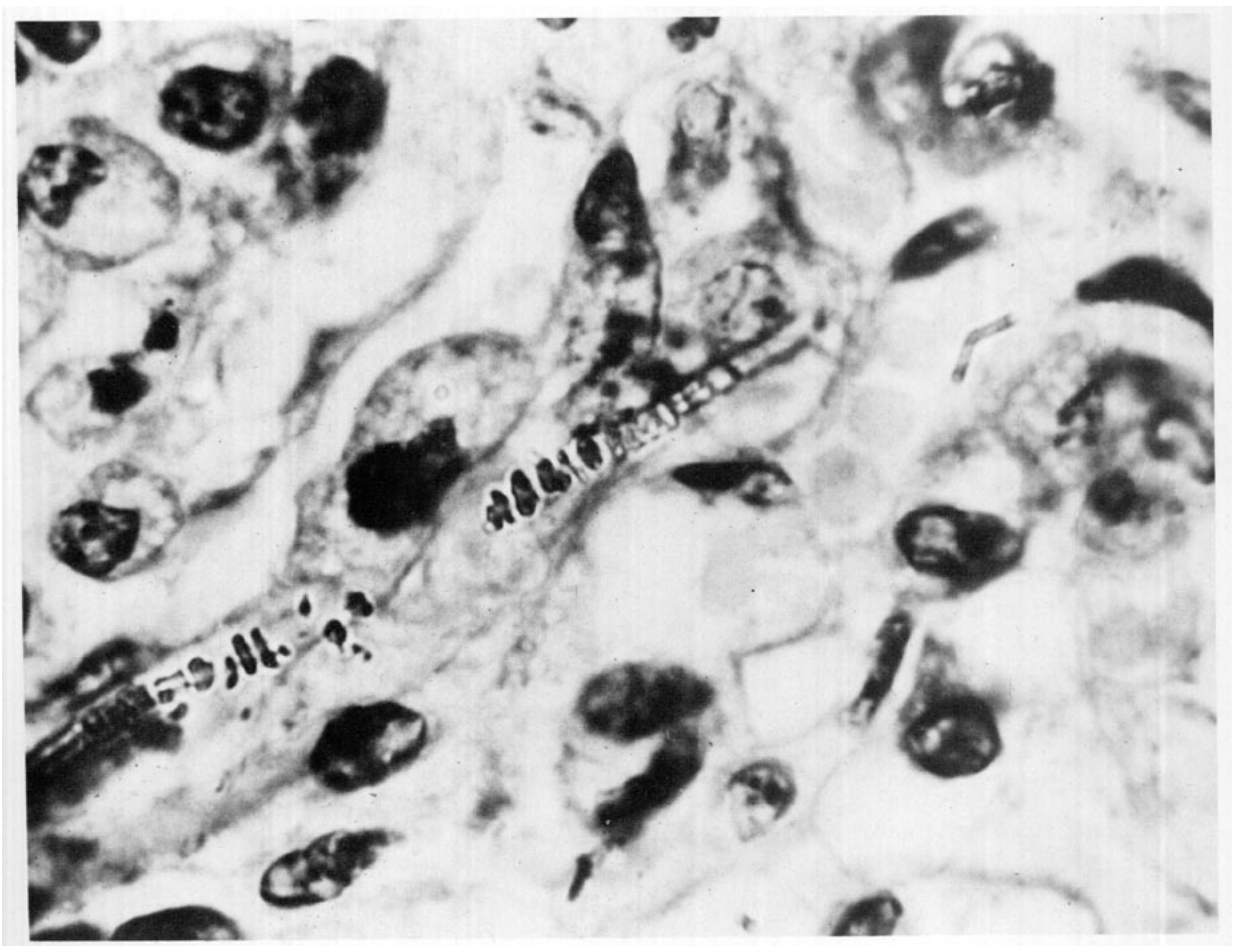

FIGURE 3. Disintegrating asbestos body. Guinea pig inhaled anthophyllite for 50 hr and was killed 15 months later. Weigert and Van Gieson. 250×.

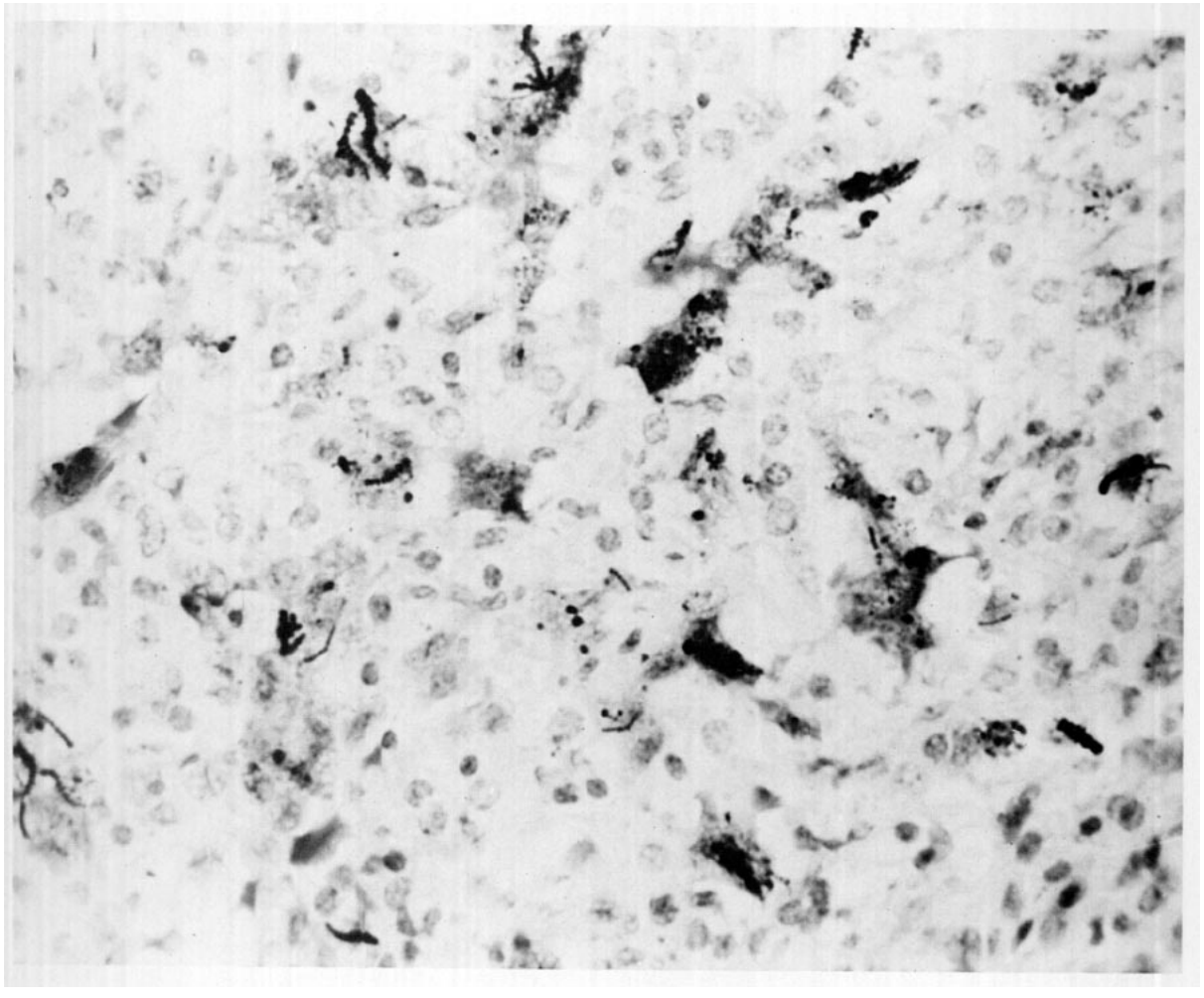

FIGURE 4. Fragments of asbestos body phagocytized and dissolved by macrophages. The resulting iron-containing solution stains intensely blue with Perls' reagent. Guinea pig inhaled chrysotile for 24 hr and was killed 18 months later. Perls-hematoxylin-eosin. 66×.

Perhaps the study of this protective mechanism may prove to be more rewarding than the study of the pathological processes.

Since the process of detoxication of the asbestos fiber and the formation of the asbestos body is initiated by the ingestion of the fiber by a macrophage, any factor that reduces the availability of macrophages would be expected to reduce the number of asbestos bodies formed. Assuming a limited availability of macrophages, the inhalation of dust particles that are cytotoxic, or even a high dose of an inert dust, should reduce the number of macrophages available for the detoxication of asbestos fibers. The formation of asbestos bodies could therefore be used as a measure of macrophage activity.

Silica dust is cytotoxic. In exploratory experiments, SPF guinea pigs have inhaled silica dust for 200 hr, then these animals, together with controls that received no silica, inhaled anthophyllite asbestos for 10 hr only. All the animals were killed 6 weeks after the inhalation of asbestos and sections were made of the lungs which were stained with eosin and Perls' reagent to pick out the asbestos bodies. The asbestos bodies in each section were counted by scanning six equally spaced strips 230 μm wide. The strips were traced on an enlarged photograph of the whole section and the length

of tissue traversed was measured. From these measurements the number of asbestos bodies in a square millimeter of tissue section was calculated.

When asbestos fibers were inhaled into a lung in which the number of active macrophages had been reduced by silica, the formation of asbestos bodies was almost completely inhibited, although many uncoated asbestos fibers were visible in the sections (Fig. 5). This fact is emphasized by the counts given in Table 1. Long asbestos fibers will not then be removed from the lung by the usual protective mechanism, and they will presumably remain pathogenic.

In another experiment, SPF guinea pigs inhaled carbon dust in high concentrations for 400 hr then anthophyllite asbestos for 10 hr. In sections cut from the lungs of animals killed 4 weeks after inhaling the asbestos, the carbon particles were seen to be almost entirely intracellular, packed into the scavenging macrophages. Some asbestos fibers were also in macrophages but others were extracellular. In sections of the control lungs from the animals that had only received asbestos, many asbestos bodies were visible. Very few asbestos bodies were found in the lungs that also contained carbon (Table 1). This observation is relevant to studies on progressive massive fibrosis, the pneumoconiosis of coal miners.

Apparently, the number of long asbestos fibers that are detoxicated by coating, converted into asbestos bodies with subsequent fragmentation, is largely reduced by a dust that destroys macrophages or even by a dust that is regarded as "safe" if the latter is inhaled in large quantity. Presumably, infection would act in a similar manner to reduce the number of macrophages available for removing asbestos particles, and a similar role may be envisaged for the very high concentration of particles found in tobacco smoke. An attempt is being made to demonstrate this.

Table 1. Asbestos bodies formed from asbestos fibers in lungs containing silica and carbon dust compared with control counts on lungs containing only asbestos.

Animal no.	Lung	Survival time, days	Asbestos bodies, count/mm² lung section × 10		
			Carbon	Quartz	Control
1	Right	28	4		
1	Left	28	5		
2	Right	28	3		
2	Left	28	1		
3	Right	28			37
3	Left	28			23
4	Right	28			37
4	Left	28			17
5	Right	41		0.3	
5	Left	41		0	
6	Right	41			8
7	Left	41			16
8	Right	57		0.7	
8	Right	57		0	
9	Right	57			47
10	Right	57			27

[a] Survival time is the time elapsing between inhalation of asbestos and death.

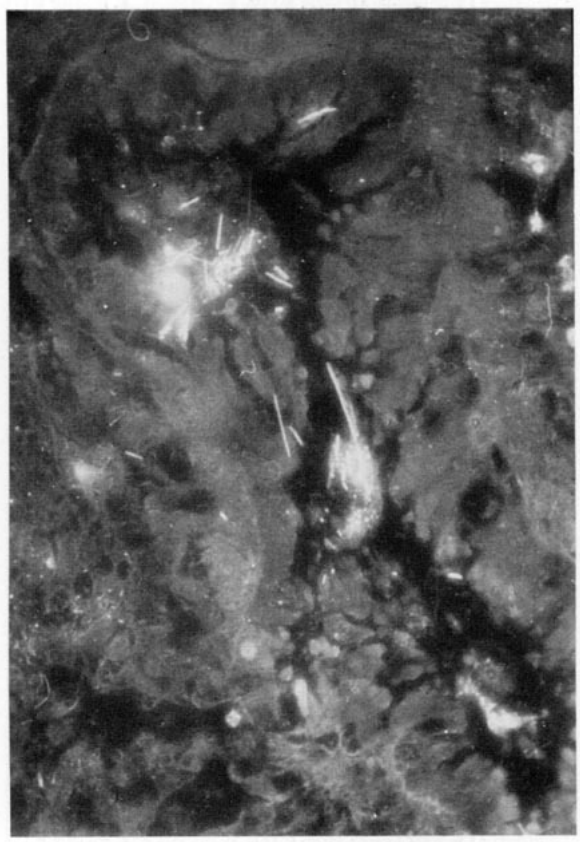

FIGURE 5. Unaffected asbestos fibers lying in the lung of a guinea pig that first inhaled silica dust. None of these fibers is coated, whereas many asbestos bodies were found in control animals that inhaled asbestos from the same atmosphere for the same time. Eosin; phase contrast. 216×.

It has already been shown *(10)* that the rate at which fibers are coated to form asbestos bodies depends on the composition of the fiber; for example, chrysotile asbestos and glass fiber are coated and fragmented more rapidly than crocidolite asbestos. This implies that the rate could be affected by coating the fiber and that perhaps some substances might accelerate the detoxication process. Among the substances used in a preliminary survey, poly(2-vinylpyridine 4-oxide) was found to be ineffective, but the results obtained with an alkylpyridine 1-oxide are certainly worth pursuing.

We have concluded that, while it is important to study by animal experimentation the pathology, particularly carcinogenesis, that results from the presence of asbestos in tissues, some means must be devised for expressing the severity of early stages in the tissue changes that is better than the subjective assessment so far employed. Possible quantitative methods are being investigated. But, in parallel with this work, a study of the protective role of the macrophage, which can be assessed objectively, appears to offer another line of attack that has given encouraging results.

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